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WO 01/49338 A1

(54) Title: CONTROLLED DELIVERY OF THERAPEUTIC AGENTS BY INSERTABLE MEDICAL DEVICES

(57) Abstract: A medical device and method for transportation and release of a therapeutic agent into a mammalian body are disclosed. The medical device is coated with alternating layers of a negatively charged therapeutic agent and a cationic polyelectrolyte, following a controlled adsorption technique. The method is simple, with minimal perturbation to the therapeutic agent and uses clinically acceptable biopolymers such as human serum albumin. The amount of the therapeutic agent that can be delivered by this technique is optimized by the number of the layers of the therapeutic agent adsorbed on the surface of medical device. There is a washing step between alternate layers of the therapeutic agent and cationic polyelectrolyte carrier, so that the amount of the therapeutic agent on the insertable medical device represents the portion that is stably entrapped and adsorbed on to the medical device. The insertable medical device and method according to this invention are capable of reproducibly delivering therapeutic agent to a site in a mammalian body, and allow for a highly reproducible and controllable release kinetics of the therapeutic agent.

One potential drawback to conventional localized drug administration is the uncontrolled manner at which the drug or drug solution is released from the delivery device. It is often desired, if not necessary, to control and/or lengthen the time period over which the drug is released. For example, it might be advantageous to lengthen the release time from seconds to minutes, or from minutes to hours, days, or even weeks. Exceptionally long release times as long as several months are often desired, for example, where the drug is released from an implanted device such as a stent. Moreover, it is often desired to control the release rate of the drug over prolonged periods of time.

Gene therapy provides an alternative approach to combating many intractable cardiovascular diseases. A site-specific delivery of the genetic vectors to minimize systemic complications is crucial for the therapeutic potential of this approach to be realized. Advances in interventional radiology and innovative designs in balloon angioplasty and stents have raised that possibility.

The invention disclosed herein solves the potential drawbacks to the drug delivery methods and instruments of the prior art by providing novel apparatus and methods for the transfer of therapeutic agents, such as therapeutic genes, to internal body sites. The apparatus of the invention may be guided to diseased or deficient organs, or other lesions, and deliver the therapeutic agent in a targeted and controlled manner.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method of delivering a negatively charged therapeutic agent to a target location within a mammalian body. The method comprises the steps of applying a multiplicity of alternating layers of at least one cationic polyelectrolyte carrier and a multiplicity of layers of a negatively charged therapeutic agent to at least one surface of an insertable medical device. A washing step is employed between application of the cationic polyelectrolyte and the negatively charged therapeutic agent. The medical device is delivered to a target site within the body, and upon reaching the target site the negatively charged therapeutic agent is released into the target site. The negatively charged therapeutic agent remains qualitatively and quantitatively intact during the stages of coating, washing, delivery and release.

In a preferred embodiment of this invention, the at least one cationic polyelectrolyte carrier is human serum albumin, gelatin, chitosan or a combination thereof.

FIG. 2 is a graph showing the relationship between the number of DNA layers and the amount of DNA adsorbed on the surface of a medical device. Released DNA was measured against the number of layers of DNA coatings on the surface of the medical device.

FIG. 3 is a photographic image of a DNA coated balloon catheter (ethydium bromide stained) before and after the DNA release. 1: DNA coated balloon catheter (stained with ethydium bromide); 2: DNA coated balloon catheter after *in vitro* release stained with ethydium 20 bromide; and 3: control uncoated balloon catheter .

FIG. 4 is a graph showing release kinetics studies using gelatin or chitosan coatings

■ without outer coating

◊ with gelatin coating (2%)

□ with chitosan coating (10 ppm)

◇ with chitosan coating (20 ppm)

◘ with chitosan coating (40 ppm)

FIG. 5 is a histogram showing transfection rate of HEK 293 cells with DNA released from a balloon medical device. Columns 1-6 each represents the following:

1: pRE-Luc + Lipofectamine; 2: pRE-Luc released from the balloon + Lipofectamine; 3: pRE-Luc; 4: DNA+ Chitosan coated surface; 5: DNA+ gelatin coated surface; 6: DNA coated surface.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the following terms are defined as follows:

"Therapeutic agent" as used herein includes any compounds or compositions that induce a biological/medical reaction *in vitro, in situ, or in vivo* settings.

"***Negatively charged therapeutic agent*** " as used herein, encompasses therapeutic agents

that are negatively charged, either naturally or synthetically. A negative charge may be added by any known chemical means or biological means *(i.e.*, addition or deletion of functionalities, substitutions, or mutations).

" ***Therapeutic polynucleotide"*** as used herein includes nucleic acids with and without carrier vectors, compacting agents, virus, polymers, proteins, or targeting sequences.

" ***Stenosis"*** refers to a stricture of any bodily canal.

" ***Stent"*** refers to any tubular structure used to maintain or support a bodily orifice or cavity.

and release.

The medical device used in this invention is any insertable medical device, including, for example, stents, catheters, or balloon catheters. A preferred medical device for use with the present invention is a balloon catheter. The medical device of this invention can be used, for example, in any application for treating, preventing, or otherwise affecting the course of a disease or tissue or organ dysfunction. For example, the medical instrument of the invention can be used to induce or inhibit angiogenesis, or to prevent or treat restenosis, cardiomyopathy, or other dysfunction of the heart, and is particularly applicable to angioplasty treatment.

Additionally, the method and medical device described herein can be used, for example, in treating cystic fibrosis or other dysfunction of the lung, for treating or inhibiting malignant cell proliferation, for treating any malignancy, and for inducing nerve, blood vessel or tissue regeneration in a particular tissue or organ.

Specific examples of the negatively charged therapeutic agent used in conjunction with the present invention includes, for example, any negatively charged compounds or compositions that are negatively charged, either naturally or synthetically by means of known chemical methods. In particular, the terms "therapeutic agents" and "drugs" are used interchangeably herein and include pharmaceutically active compounds and compositions, polynucleotides with and without carrier vectors such as lipids, compacting agents (such as histones), virus, polymers, proteins, and the like, with or without targeting sequences.

Specific examples of the polynucleotide used in conjunction with the present invention include, for example, oligonucleotides, ribozymes, anti-sense oligonucleotides, DNA compacting agents, gene/vector systems (*i.e.*, any vehicle that allows for the uptake and expression of nucleic acids), nucleic acids (including, for example, recombinant nucleic acids; naked DNA, cDNA, RNA; genomic DNA, cDNA or RNA in a non-infectious vector or in a viral vector and which further may have attached peptide targeting sequences; antisense nucleic acid (RNA or DNA); and DNA chimeras which include gene sequences and encoding for ferry proteins such as membrane trans locating sequences ("MTS") and herpes simplex virus-l ("VP22"),and constitutive housekeeping genes which are theoretically expressed in all cell types.

Non-limiting examples of virus vectors or vectors derived from viral sources include adenoviral vectors, herpes simplex vectors, papilloma vectors, adeno-associated vectors, retroviral vectors, and the like. The use of adenovirus is particularly preferred.

DNA coding for tRNA or rRNA to replace defective or deficient endogenous molecules. The polynucleotides of the invention can also code for therapeutic proteins or polypeptides. A polypeptide is understood to be any translation product of a polynucleotide regardless of size, and whether glycosylated or not. Therapeutic proteins and polypeptides include as a primary example, those proteins or polypeptides that can compensate for defective or deficient species in an animal, or those that act through toxic effects to limit or remove harmful cells from the body.

In addition, the polypeptides or proteins, DNA of which can be incorporated, include without limitation, angiogenic factors and other molecules competent to induce angiogenesis, including acidic and basic fibroblast growth factors, vascular endothelial growth factor, hif-l, epidermal growth factor, transforming growth factor α and β, platelet -derived endothelial growth factor, platelet-derived growth factor, tumor necrosis factor α, hepatocyte growth factor and insulin-like growth factor; growth factors; cell cycle inhibitors including CDK inhibitors; anti- restenosis agents, including p15, p16, p18, p19, p21, p27, p53, p57, Rb, nFkB and E2F decoys, thymidine kinase ("TK ") and combinations thereof and other agents useful for interfering with cell proliferation, including agents for treating malignancies; and combinations thereof. Still other useful factors, which can be provided as polypeptides or as DNA encoding these polypeptides, include monocyte chemoattractant protein ("MCP-I "), and the family of bone morphogenic proteins ("BMP's"). The known proteins include BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 (Vgr-1), BMP- 7 (OP-I),BMP-8, BMP-9, BMP-10, BMP-11, BMP-12,BMP-13,BMP- 14, BMP-15, and BMP-16. Currently preferred BMP's are any ofBMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP- 7. These dimeric proteins can be provided as homodimers, heterodimers, or combinations thereof, alone or together with other molecules. Alternatively or, in addition, molecules capable of inducing an upstream or downstream effect of a BMP can be provided. Such molecules include any of the "hedgehog" proteins, or the DNA' s encoding them.

The amount of polynucleotide adsorbed is an effective expression-inducing amount. As used herein, the term "effective expression-inducing amount" means that amount of the polynucleotide that effectuates expression of a gene product encoded by such polynucleotide. Means for determining an effective expression-inducing amount of a polynucleotide are well known in the art. For example, an effective expression-inducing amount of the polypeptide of this invention is from about 0.3 to about 10 μg /cm^2/layer, preferably from about 0.5 to about 0.9 μg /cm^2/layer. The amount of polynucleotide adsorbed onto the, surface of the medical

achieved before the release occurred. Chitosan, a natural polysaccharide derived from crab shells, was used and shown to serve this purpose. Other polymers can also be used to fine-tune the release kinetics of the therapeutic agent from the coated surface. For example, with a thin coaating of condensed gelatin or chitosan, a short lag time of about 1-2 minutes is achieved before release occurs . Without the use of gelatin or chitosan, 100% of the DNA is released at physiological pH within minutes.

Organs and tissues that are treated by the methods of the present invention include any mammalian tissue or organ, whether injected *in vivo* or *ex vivo*. Non-limiting examples include

the heart, lung, brain,. liver, skeletal muscle, smooth muscle, kidney, bladder, intestines, stomach, pancreas, ovary , prostate. cartilage and bone.

The negatively charged therapeutic agents. according to the invention, can be used, for example, in any application for treating, preventing, or otherwise affecting the course of a disease or tissue or organ dysfunction. For example, the methods of the invention can be used to induce or inhibit angiogenesis, as desired, to prevent or treat restenosis, to treat a cardiomyopathy or other dysfunction of the heart, for treating cystic fibrosis or other dysfunction of the lung, for treating or inhibiting malignant cell proliferation, for treating any malignancy, and for inducing nerve, blood vessel or tissue regeneration in a particular tissue or organ. Particularly, the negatively charged therapeutic agents of this invention are used preferably in angioplasty . Having now fully described the invention, the same would be more readily understood by reference to specific examples which are provided by way of illustration, and not intended to be limiting of the invention, unless herein specified.

Example* 1. *Effect of surfaces and polyelectrolytes on the DNA release

Multilayered films of DNA were built up on various negatively charged, neutral, and positively charged surfaces, by spraying or dipping. The DNA adsorbed by HSA or gelatin was released quickly whereas, due to the hydrophobicity of chitosan at neutral pH, the DNA adsorbed by chitosan was released very slowly. The result of this experiment is tabulated in Table I below. Table 1 shows natural polymers, as polyelectrolytes, are coated onto several surfaces, which surfaces were modified by different substrates. When different surfaces were dipped into a slightly acidic solution containing a polynucleotide, the positively charged coated surface induced adsorption of the polynucleotide (*i.e.*, adsorption was driven by the charged interaction). Successive layering of the surface with polyelectrolyte and DNA can be repeated as many times as needed to maximize the amount of DNA adsorbed to the surface.

Example 4. Biological activity of the released DNA

The biological activity of DNA, released from the surface of a coated medical device, was investigated by transfecting HEK 293 cells *in vitro*. The result of this study, as shown in Fig. 5, indicates that the released DNA was still biologically active. A comparison between columns 1 and 2 of Fig. 5 shows that the DNA released from the medical device coated with gelatin or chitosan, similar to the naked DNA, had a high transfection efficiency. Occasionally, cationic gelatin complexes with DNA in the soluble form and transfects cells in culture better than naked DNA.

Example 5. Feasibility of delivering adenovirus

Using similar adsorption technique with gelatin as the polycation, ^{125}I labeled recombinant adenovirus, encoding the Lac Z gene, was adsorbed onto a balloon surface. The result of this experiment is shown in Table 2 below. Table 2 indicates that the amount of plaque forming units and virus particles of adenovirus, released or remained on the balloon after release delivery, constitutes a major portion of the total amount of virus found on the surface of the balloon.

Table 2. Feasibility of delivering adenovirus

	Readings	Total amount of virus (pfu)	(particles)	Amount of adenovirus (pfu/cm^2)	(vp/ cm^2)
Calibration Standard	538.5 ±52	$6.4 \pm 0.6 \times 10^7$	$1.6 \pm 0.2 \times 10^9$	-	-
Released adenovirus	47±9.5	$5.6 \pm 1.1 \times 10^6$	$1.4 \pm 0.3 \times 10^8$	$4.0 \pm 0.8 \times 10^6$	$1.0 \pm 0.2 \times 10^8$
Virus remained balloon after release	168.5±58	$2.0 \pm 0.7 \times 10^7$	$5.0 \pm 1.7 \times 10^8$	$1.4 \pm 0.5 \times 10^7$	$3.5 \pm 1.2 \times 10^8$

Table 2 shows the amount of adenovirus released in 60 minutes in 10% serum culture media from a 10-layered balloon.

Example 6. DNA delivery via negatively charged polystyrene (PS) surface

Carboxylated polystyrene (PS) wells were treated with 360 µl (per well) of 0.1 % human serum albumin (HSA) in 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) at room temperature (RT) overnight. The wells were washed thoroughly with water and then treated with 360 µl (per well) of DNA (247 µg/ml) in 25 mM HAc- NaAc/25 mM Na_2SO_4 buffer (pH 4.0) at RT for 0.5 hr. The wells were washed once with 360 µl (per well) of 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) and then treated again with 360 µl of 0.1% HSA in 25 mM HAc- NaAc/25 mM Na_2SO_4 buffer (pH 4.0) at RT for 0.5 hr. The wells were

Na_2SO_4 buffer (pH 4.0) at RT for 0.5 hr. The balloons were washed once with 5 ml of 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) and then treated again with 5 ml of 0.1% HSA in 25mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) at RT for 0.5 hr. Balloons were washed once with 5 ml of 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) and then treated again with 5 ml of DNA (141 μg/ml) in 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) at RT for 0.5 hr. The preceding washing steps were repeated until 4 DNA layers were adsorbed. Results are tabulated in Table 5 below.

Table 5. DNA delivery on two balloons.

	A	B
Readings (ng/ml)	55	54
Released DNA (μg)	2.42	2.376
Released DNA (μg/ cm^2)	1.287	1.264

A: Balloon was washed once with 5 ml of 25 mM HAc-NaAc/25 mM Na_2SO_4 buffer (pH 4.0) and then dipped in 4 ml of I X PBS at RT for 0.5hr.

B: Balloon was not washed and directly dipped in 4 ml of 1 X PBS at RT for 0.5 hr. The amount of DNA released in PBS was determined (200 μl was taken into 2 ml of test solution).

Example 9. Transfection of HEK 293 cells in vitro

In a twelve-well tissue culture plate, 8 x 10^4 HEK 293 cells per well were seeded in 1 ml of the appropriate complete growth medium (10% serum) and incubated at 37°C in a CO_2 incubator for 1 day. The culture medium was then removed and the transfection medium was added to the cells. The cells were then divided into 6 different groups:

Group 1: 2 μg of DNA (Luci) and 2 μl of lipofectamine in 1 ml of serum-free medium; Group 2: Released DNA (Luci, 20 layers) and 2 μl of lipofectamine in 1 ml of serum-free medium. Group 3: 2 μg of DNA (Luci) in 1 ml of serum-free medium. Group 4: Released DNA (Luci, 20 layers) with an outermost chitosan coating. Group 5: Released DNA (Luci, 20 layers) with an outermost gelatin coating. Group 6: Released DNA (Luci, 20 layers). The cells were incubated at 37°C in a CO_2 incubator for three days. The media was removed from the cells and the cells were rinsed once with 1 X PBS. Cell Culture Lysis (200 μl) was added at the concentration of 1 X Reagent per well to cover the cells. The cells were

We Claim:

1. An implantable medical device comprising an implantable medical device having a coating on at least one portion of at least one surface, said coating comprising:

an inner layer of a cationic polyelectrolyte carrier: and

a layer of at least one negatively charged therapeutic agent adsorbed onto said inner layer of cationic polyelectrolyte carrier: and

optionally: an additional layer or layers of cationic polyelectrolyte carrier and an additional layer or layers of at least one negatively charged therapeutic agent adsorbed onto said additional layer or layers of cationic polyelectrolyte carrier, wherein said additional layer or layers of polyelectrolye carrier and said additional layer or layers of negatively charged therapeutic agent alternate.

2. The medical device of claim 1. further comprising an outermost layer of a cationic polyelectrolyte carrier which is the same or different from the inner or additional layer or layers of cationic polyelectrolyte carrier.

3. The medical device of claim 2, wherein the outermost layer of cationic polyelectrolyte carrier is more hydrophobic and/or more cationic than at least one inner or additional layer or layers of polyelectrolyte carrier.

4. The medical device of claim 1, wherein at least one inner or additional layer of cationic polyelectrolyte carrier comprises human serum albumin, gelatin, chitosan, or a combination thereof.

5. The medical device of claim 1, wherein the medical device comprises a stent, a catheter, a balloon catheter. or a combination thereof.

6. The medical device of claim 1, wherein at least one of the one or more negatively charged therapeutic agent comprises at least one agent selected from the group consisting of anti-thrombogenic agents, antioxidants, angiogenic agents, anti-angiogenic agents, agents capable of blocking smooth muscle cell proliferation, anti-inflammatory agents, calcium entry blockers. antineoplastic agents, antiproliferative agents, anti-mitotic agents, anti-microbials, anesthetic agents. nitric oxide donors, anti-coagulants, vascular ell growth promoters, vascular cell growth inhibitors, cholesterol lowering agents, vasodilating agents, agents which interfere with endogenous vasoactive mechanisms, agents that protect against cell death, cell cycle inhibitors, anti-restenosis agents, agents for treating malignancies, bone morphogenic proteins, and polynucleotides encoding such agents.

7. The medical device of claim 1, wherein at least one of the one or more therapeutic agent

of blocking smooth muscle cell proliferation, anti-inflammatory agents, calcium entry blockers, antineoplastic agents, antiproliferative agents, anti-mitotic agents, anti-microbials, anesthetic agents, nitric oxide donors, anti-coagulants, vascular ell growth promoters, vascular cell growth inhibitors. cholesterol lowering agents, vasodilating agents. agents which interfere with endogenous vasoactive mechanisms. agents that protect against cell death, cell cycle inhibitors, anti-restenosis agents, agents for treating malignancies, bone morphogenic proteins, and polynucleotides encoding such agents.

16. The method of claim 10. wherein at least one of the one or more therapeutic agent comprises rapamycin.

17. The method of claim 10, wherein at least one of the one or more therapeutic agent comprises paclitaxel.

18. The method of claim 10. wherein at least one of the one or more therapeutic agent comprises a polynucleotide encoding a therapeutic molecule, wherein said polynucleotide is inserted into an adenovirus vector.

19. A medical device comprising a negatively charged therapeutic agent adsorbed on the surface thereof and produced by a process comprising:

(a) coating at least one portion of at least one surface a medical device with a cationic polyelectrolyte carrier to form an inner layer of cationic polyelectrolyte carrier;

(b) washing the layer of cationic polyelectrolyte carrier with a washing solution;

(c) adsorbing one or more negatively charged therapeutic agent onto the layer of cationic polyelectrolyte carrier to form a layer of therapeutic agent; and optionally

(d) washing the layer of therapeutic agent with a washing solution and repeating steps (a) through (c) one or more times to form multiple layers of cationic polyelectrolyte carrier and therapeutic agent until a desired amount of therapeutic agent has been adsorbed onto the medical device.

20. The medical device of claim 19, wherein the process further comprises the step of coating the outermost layer of therapeutic agent with an outermost layer of a cationic polyelectrolyte carrier which is the same or different from the inner layer or multiple layers of cationic polyelectrolyte carrier.

21. The method of claim 20, wherein the outermost layer of cationic polyelectrolyte carrier is more hydrophobic and/or more cationic than at least one of the inner layer or multiple layers of cationic polyelectrolyte carrier.

carrier and therapeutic agent until a desired amount of therapeutic agent has been adsorbed onto the medical device.

29. The method of claim 28, further comprising the step of coating the outermost layer of therapeutic agent with an outermost layer of a cationic polyelectrolyte carrier which is the same or different from the inner layer or multiple layers of cationic polyelectrolyte carrier.

30. The method of claim 29, wherein the outermost layer of cationic polyelectrolyte carrier is more hydrophobic and/or more cationic than at least one of the inner layer or multiple layers of cationic polyelectrolyte carrier.

31. The method of claim 28, wherein at least one of the inner layer or multiple layers of cationic polyelectrolyte carrier comprises human serum albumin, gelatin, chitosan, or a combination thereof.

32. The method of claim 28, wherein the medical device comprises a stent, a catheter, a balloon catheter, or a combination thereof.

33. The method of claim 28, wherein at least one of the one or more negatively charged therapeutic agent comprises at least one agent selected from the group consisting of anti-thrombogenic agents, antioxidants, angiogenic agents, anti-angiogenic agents, agents capable of blocking smooth muscle cell proliferation, anti-inflammatory agents, calcium entry blockers, antineoplastic agents, antiproliferative agents, anti-mitotic agents, anti-microbials, anesthetic agents, nitric oxide donors, anti-coagulants, vascular ell growth promoters, vascular cell growth inhibitors, cholesterol lowering agents, vasodilating agents, agents which interfere with endogenous vasoactive mechanisms, agents that protect against cell death, cell cycle inhibitors, anti-restenosis agents, agents for treating malignancies, bone morphogenic proteins, and polynucleotides encoding such agents.

34. The method of claim 28, wherein at least one of the one or more therapeutic agent comprises rapamycin.

35. The method of claim 28, wherein at least one of the one or more therapeutic agent comprises paclitaxel.

36. The method of claim 28, wherein at least one of the one or more therapeutic agent comprises a polynucleotide encoding a therapeutic molecule, wherein said polynucleotide is inserted into an adenovirus vector.

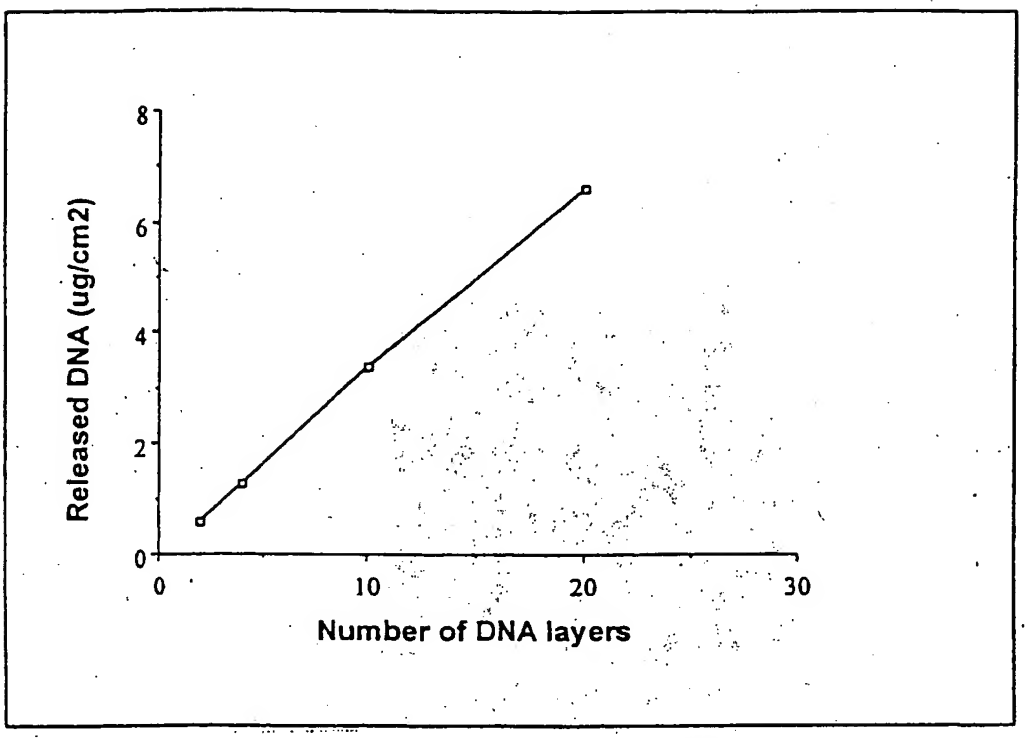
37. The method of claim 28, wherein the target location comprises at least one location selected from the group consisting of brain, heart, liver, skeletal muscle, smooth muscle, kidney, bladder, intestines, stomach, pancreas, ovary, prostate, cartilage, bone, lung, blood

therapeutic agent comprises at least one agent selected from the group consisting of anti-thrombogenic agents, antioxidants, angiogenic agents, anti-angiogenic agents, agents capable of blocking smooth muscle cell proliferation, anti-inflammatory agents, calcium entry blockers, antineoplastic agents, antiproliferative agents, anti-mitotic agents, anti-microbials, anesthetic agents, nitric oxide donors, anti-coagulants, vascular ell growth promoters, vascular cell growth inhibitors, cholesterol lowering agents, vasodilating agents, agents which interfere with endogenous vasoactive mechanisms, agents that protect against cell death, cell cycle inhibitors, anti-restenosis agents, agents for treating malignancies, bone morphogenic proteins, and polynucleotides encoding such agents.

44. The method of claim 38, wherein the clinical disease or condition comprises restenosis or angiogenesis and at least one of the one or more therapeutic agent comprises rapamycin.

45. The method of claim 38, wherein the clinical disease or condition comprises a malignancy or malignant cell growth and at least one of the one or more therapeutic agent comprises paclitaxel.

46. The method of claim 38, wherein the target location comprises at least one location selected from the group consisting of brain, heart, liver, skeletal muscle, smooth muscle, kidney, bladder, intestines, stomach, pancreas, ovary, prostate, cartilage, bone, lung, blood vessel, ureter, urethra, ovary, testes, malignant growth, or benign growth.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2
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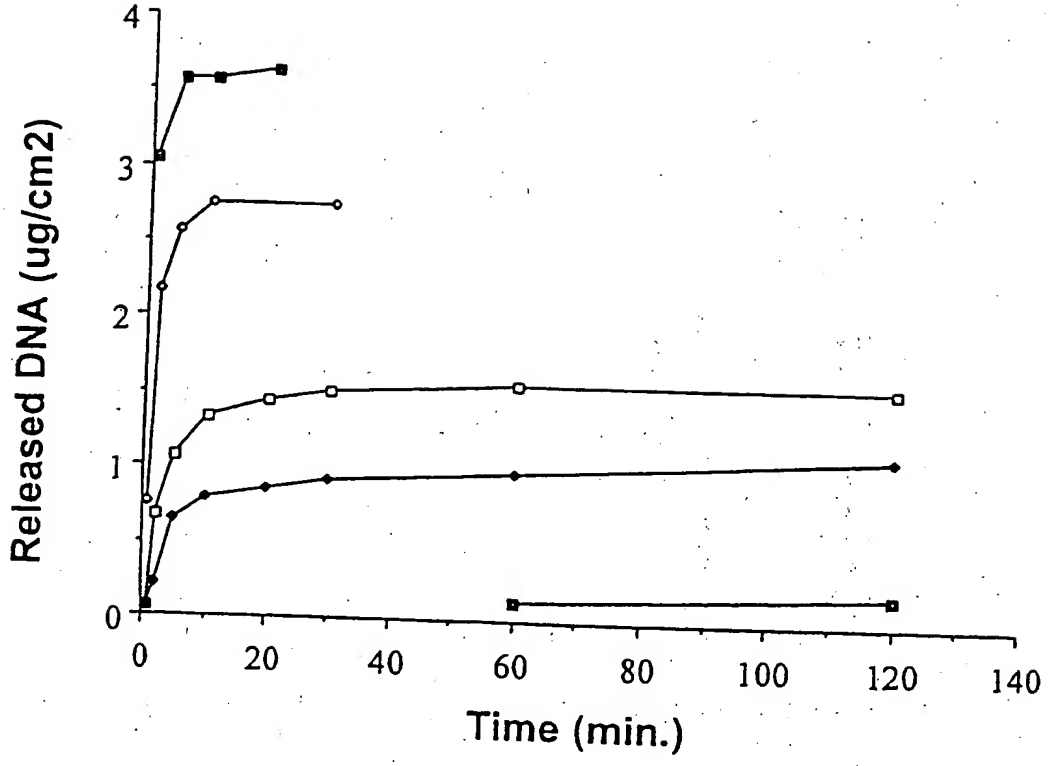
20
30
Number of DNA layers


Released DNA (ug/cm2)
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4
0
20
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60
80
100
120
140
Time (min.)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/00025

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L29/16 A61L31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61M C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 08729 A (BOSTON SCIENT CORP) 25 February 1999 (1999-02-25) page 2, line 26 -page 3, line 6 page 3, line 18 -page 6, line 23 examples 1-9 claims 1,3,4,11,18 --- -/--	1-6,8, 19-24, 26, 28-33, 35, 37-43, 45,46

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2001	22/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Menidjel, R

Form PCT/ISA/210 (second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 28-46 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy